



## The Effects of Licorice Root Powder (*Glycyrrhiza glabra*) on Performance, Serum Parameters, Egg Yolk Cholesterol and Antioxidant Capacity of Laying Japanese Quail

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### ABSTRACT

This study was conducted with the objectives to determine the effects of licorice root powder (*Glycyrrhiza glabra*) on performance, serum parameters, egg yolk cholesterol and antioxidant capacity in laying Japanese quail. Two hundred and forty 10-wk-old Japanese quails were randomly assigned to four dietary groups, each one four times replicated with fifteen quails per dietary groups. Control group fed the basal diet; other groups were fed basal diet supplemented with 0, 0.5, 1.0 and 1.5% licorice root powder. There were no significant differences in terms of final live weight, feed intake, feed conversion ratio, egg weight, serum cholesterol, triglyceride and High-Density Lipoprotein (HDL) concentration between treatment groups. Egg production was higher in control group and 0.5% licorice root powder supplemented groups. 1.0% licorice root powder supplemented group had lower egg production rate. Licorice root powder supplementation decreased Low Density Lipoprotein (LDL) concentration and increased glucose concentration significantly. Licorice root powder supplementation increased total antioxidant status (TAS) and reduced total oxidant status (TOS) and oxidative stress index (OSI). It was concluded from this study that supplementation of licorice root powder at the level of 0.5, 1.0 and 1.5% to the feed of laying quails had no adverse effects on performance. Licorice root powder supplementation can be used to reduce cholesterol level and increase antioxidant status in quails.

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### Introduction

Many of the medicinal and aromatic plants present in the nature are natural and reliable, they have been widely used for medical purposes and spices for centuries due to their various pharmacological effects. These plants have various functional properties due to the bioactive components they contain (Kohlert et al., 2000). When plants and extracts are consumed by humans and animals, they are regarded as safe additives that are safe from chemical point of view. The use of medicinal and aromatic plants in animal nutrition has increased, in order to replace the use of antibiotics especially after the ban of antibiotic feed additives within the European Union countries in 2006 (Franz et al., 2010).

Licorice root (*Glycyrrhiza glabra*) is among the oldest and most widely known medicinal plants in the world. *Glycyrrhiza glabra* is a perennial herb, native to central and South-Western Asia, as well as to the Mediterranean

region and cultivated in temperate and sub-tropical regions of the world, including Europe and Asia. The root, dried and processed, is called *licorice* and has a characteristic odor and sweet taste (Fiore et al., 2008).

Licorice root, *Glycyrrhiza* genus, belongs to the Fabaceae family, and there are about 30 species spread all over the world (Shibata, 2000). The roots of the plant are widely used in medicinal and industrial environments in many countries. Despite the fact that licorice plant has been used for many years in traditional herbal medicine, its composition and pharmacologically active compounds have been revealed through modern analytical techniques and scientific research conducted over the last 25-30 years (Asl and Hosseinzadeh, 2008). The majority of these bioactive compounds are triterpene saponins (4-20%) and various types of phenolic compounds (Fiore et al., 2008; Tan et al., 2010).

Glycyrrhizin and flavonoids, such as liquiritin, isoliquiritin and their aglycones, have been reported as the major constituents of licorice and they are perceived as the active principles responsible for its pharmacological efficacy (Zhang and Ye, 2009). Glabridin which is the main isoflavonoid compounds of the plant has been reported to have potent antioxidant activity (Shibata, 2000).

The effects of glycyrrhizin (glycyrrhizin) and glycyrrhetic (glycyrrhetic) acid as the main compounds of triterpene saponins have been demonstrated as antioxidant (Ju 1989; Vaya et al., 1997; Doğan, 2004), anti-inflammatory (Yokota et al., 1998), anti-viral (Fiore et al., 2008), anti-allergic, anticarcinogenic and immunomodulatory with the results of clinical and experimental studies (Shibata, 2000; Asl and Hosseinzadeh, 2008). Other important effects of glycyrrhizin and glycyrrhetic acid have been reported to have cardioprotective, hepatoprotective and plasma lipid-lowering effects (Fuhrman et al., 2002, Nakagawa et al., 2004, Visavadiya and Narasimhacharya, 2006).

Vaya et al. (1997) investigated the effect of antioxidant substances of licorice root on the LDL oxidation. They have identified 7 isoflavan substances in the root. (Hispaglabridine, Glabridin, methylglabridine isoprenylchalcone derivatives, Isoliquiritigenin, isoflavone and Formononetin). These antioxidants have been shown to be highly effective against LDL oxidation. Nitalikar et al. (2010) reported that licorice root extract have antimicrobial effects on gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). Sen et al. (2011) reported that glycyrrhizin which is one of the key components of the licorice root bring oxidative stress parameters such as Superoxide dismutase (SOD), catalase and malondialdehyde (MDA) to normal levels in diabetic mice. There is a very limited number of studies have been conducted on the use of licorice root powder or extract as supplement in laying hens or quails diet.

For this purpose, present study were investigated the effects of the licorice root powder (*Glycyrrhiza glabra*) on performance, serum parameters, egg yolk cholesterol and antioxidant capacity in laying Japanese quail.

## Material and Method

### Bird and Diet

The experimental procedures for this study were reviewed and approved by the Local Animal Care and Use Committee of Çukurova University (29.12.2014/8). Two hundred and forty 10-wk-old laying Japanese quails of similar live weight ( $370.48 \pm 2.156$  g) were randomly divided into four dietary treatments. Each treatment was consisted of 4 replicates with 15 birds in each. Quails were weight individually at the beginning and at the end of the experiment. Birds were transferred to wire cages (15 birds each) which were  $50 \times 50 \times 20$  cm. Each cage was equipped with a trough-type feeder and a nipple drinker.

Quails were fed a basal diet as a control group and other groups were fed basal diet supplemented with 0, 0.5, 1.0 and 1.5 % licorice root powder. In order to add the

licorice root powder to the basal diet, a small amount of diet was first taken and mixed with a certain amount of licorice root powder. This mixture was then added to the diet and mixed until the diet became homogenous.

The basal diet was formulated to meet the nutrient requirements of laying quails according to NRC (1994) (Table 1) Licorice root was obtained from Hatay province which is situated in southern Turkey, on the eastern Mediterranean coast. After the licorice root was powdered, it was given to the quails.

Chemical composition of licorice root was determined as 9.15% protein, 0.53% fat, 6.80% moisture, 7.70% ash, 24.48% fiber, 47.11% Carbohydrate, 1720 mg/100g Ca, 78 mg/100g P, 185.80 ppm Na, 7276 ppm K, 1224 ppm Fe (Badr et al., 2103).

Production performance such as feed intake, egg number, egg weight and bird mortality of each replicate was recorded daily. Feed conversion ratio (FCR) was calculated according to following formula:

$$FCR = \frac{\text{Feed consumption (g/week)}}{\text{Egg weight (g/week)}}$$

Egg mass was calculated by multiplying egg weight by egg production. During the 8-week experimental period 16 h light 8 h dark was applied and feed and water were given for *ad-libitum* consumption.

### Egg Yolk Cholesterol

In order to determine egg yolk cholesterol level in the experiment, 20 eggs (5 eggs from each replicate) were taken from each group at 4<sup>th</sup> and 8<sup>th</sup> weeks of experiment. The eggs were boiled for 10 minutes to ensure solidification of the egg yolk. Then the egg yolks were removed and mixed thoroughly. After homogenization, yolk samples were taken and transferred to the tubes and kept at -80°C until analysis. Fat extraction from the samples was performed according to the method developed by Boselli et al. (2001). According to this method, yolk samples were added 30/70 ratio ethanol/chloroform solution at the ratio of 2:1 and stirred at 21°C for 30 minutes. The solution obtained at the end of the filtration was filtered through a vacuum cleaner filter. Egg cholesterol levels were determined spectrophotometrically using the Boehringer Mannheim GmbH Biochemica (1989) method with the appropriate kit.

### Biochemical Analyses

At the end of the experiment, 2 birds were randomly selected per replicate from each treatment group and starved for 12 h before slaughter. Total 32 quails were slaughtered by severing the jugular vein and blood samples were taken and transferred to anticoagulant tubes.

To separate the serum, the tube was centrifuged at 2000 rpm for 10 minutes. After centrifugation, serum samples were transferred to tubes and stored at -80°C until analyzed. Glucose, triglyceride, cholesterol, HDL and LDL lipoprotein levels in serum were measured spectrophotometrically according to the protocols of commercial kits (Erba Mannheim, CZ).

Table 1 Ingredient and chemical composition of the diet

Ingredient	%	Calculated Nutrient	Composition
Maize	48.55	ME, kcal/kg	2.900.00
Soybean meal	33.95	Crude protein, %	19.78
Meat-bone meal	3.00	Crude fiber, %	2.98
Vegetable oil	5.00	Ether extract %	5.05
Lime stone	7.60	Ash %	10.02
Di calcium phosphate	1.20	Dry matter %	91.30
Salt	0.25	Available phosphorus, %	0.60
Vitamin premix*	0.10	Calcium, %	3.20
Mineral premix**	0.10	Methionine, %	0.40
DL-Methionine	0.20	Methionine & Cysteine, %	0.65
Lysine, %	0.05	Lysine, %	0.95
Total	100.00		

\*Vitamin premix in per kg of diet: 15,000 I.U. Vitamin A, 5,000 I.U. Vitamin D, 100 I.U. Vitamin E, 5 I.U. Vitamin K, 4 I.U. Vitamin B<sub>1</sub>, 10 I.U. Vitamin B<sub>2</sub>, 5 I.U. Vitamin B<sub>6</sub>, 0.03 I.U. Vitamin B<sub>12</sub>, 50 mg Vitamin C, 60 mg Niacin, 18 mg Calcium D-Pantothenic acid, 2 mg Folic Acid, 0.25 mg Biotin, \*\*Mineral premix in per kg of diet: 100 mg Manganese, 80 mg Iron, 100 mg Zinc, 10 mg Copper, 0.2 mg Cobalt, 1.5 mg Iodine, 0.2 mg Selenium.

Liver sample of quails were taken under aseptic conditions from quails that have been slaughtered and blood samples taken. Homogenates were prepared in the liver sample. The total antioxidant and oxidant capacity (TAS and TOS) parameters were investigated and the oxidative stress index was determined. TAS and TOS levels in liver samples were determined by spectrophotometer following the methodology of commercial kit (Rel Assay, TR). To prepare the homogenate from the liver samples, 0.4 g was taken from the samples in sterile environment. Homogenates were prepared by washing 2 times with PBS and then centrifuging at 4000 g for 5 minutes. And then lysis buffer including 1% triton X-100 (Merck), 50 mM HEPES pH 7.2, 10 mM EDTA, 100 mM NaH<sub>2</sub>PO<sub>4</sub> 2H<sub>2</sub>O and 8% protease inhibitor cocktail [aprotinin, phenylmethanesulfonyl fluoride (PMSF), leupeptin, sodium fluoride (Merck, EU)] was added to homogenates. Supernatants were obtained by centrifuging of detergent-insoluble cellular proteins at 12000 g for 10 minutes at 4°C. TAS and TOS analyzes were determined by using the obtained homogenate samples.

#### Total Antioxidant Status

TAS levels of liver homogenates were analyzed using commercial kits based on the principles described above (Assay Rel Diagnostics, Turkey). For this purpose, 800 µl of Reagent 1 was taken from kit and 50 µl samples were added to this. The initial measurements were made at 660 nm wavelength in the spectrophotometer and the results were recorded. Then, 125 µL of Reagent 2 was added to these test tubes and kept at room temperature for 10 minutes and measured at absorbance values of 660 nm. The obtained values were determined as mmol Trolox Eqiv./L (Erel, 2004).

#### Total Oxidant Status

TOS values in liver samples were determined according to equivalent micro molar hydrogen peroxides per liter. (Erel, 2005). TOS levels of liver homogenates were analyzed using commercial kits based on the principles described above (Assay Rel Diagnostics, Turkey). For this purpose, 1000 µl of Reagent 1 in kit was

taken and 150 µl sample was added to them and initial measurements were made with 530 nm wavelength in spectrophotometer. Subsequently, 50 µL of Reagent 2 was added to these test tubes and kept at room temperature for 10 minutes and then again were measured at 600 nm absorbance values and recorded. The values were calculated in terms of µmol H<sub>2</sub>O<sub>2</sub> Eqiv./L.

#### Oxidative Stress Index (OSI)

The effects licorice root powder on the oxidative stress and antioxidant system were determined by investigating total antioxidant and oxidant capacity (TAS and TOS) parameters and determined with oxidative stress index. The oxidative stress index is equal to the percentage of total oxidant capacity to total antioxidant capacity. The TAS value obtained in the experiment as mmol/L was converted to µmol/L and the oxidative stress index was calculated according to the following formula (Kosecik et al., 2005).

$$OSI = \frac{TOS, \mu\text{mol H}_2\text{O}_2 \text{ equiv./L}}{TAS, \mu\text{mol Trolox equivalent/L}} \times 100$$

#### Statistical Method

Statistical analysis of data was performed using the SPSS 18.0 (Statistical Package for Social Sciences) program. The data were analyzed using the One-way ANOVA variance analysis method in SPSS and the Duncan test was used to determine the statistical differences between the groups. Duncan test result P<0.05 was considered statistically significant (SPSS, 2009).

#### Results and Discussion

Results of final live weight, feed intake, feed conversion ratio, egg production and egg weight of quails are presented in Table 2. During the 1-8 wks., final live weight, feed intake, feed conversion ratio and egg weight were not affected by the licorice root powder supplementation (P>0.05). These results are in agreement with Sedghi et al. (2010a) who showed that diets supplemented with 0, 2, 4 or 6 g/kg of licorice extract were not influenced feed intake, feed conversion ratio and

egg weight in 58-wk-old laying hens. Contrary to our study, Awadein et al. (2010) who reported that dietary supplementation of licorice at the level of 0.1 and 0.5% had significant effect on feed intake, feed conversion ratio and egg weight in Mandarrah hens. They explained that feed intake and feed conversion ratio decreased and egg weight increased with dietary licorice supplementation ( $P<0.05$ ). The different results may be due to the different species of poultry or different form of licorice used in the researches. There is a very limited number of studies have been conducted on the use of licorice root powder or extract as supplement in laying hens or quails diet. Experiment on the addition of licorice root was made mostly on broiler chickens and the findings were

compared with them. Thus, in contrast to our results, Safari and Zahedi (2016) explained that 0, 0.5, 1, 1.5 and 2 g/kg *Glycyrrhiza glabra* extract significantly influenced quail body weight and feed intake during both the starter (1-21 days) and grower (21-42) periods of the study ( $P<0.05$ ), but there was not any significant effect on feed conversion ratio during the experiment ( $P>0.05$ ). Similarly, Myandoab and Mansoub (2012) investigated the effects of licorice root extract as a medicinal plants and probiotic in quails. They obtained that feed conversion ratio decreased and feed intake and average body weight gain increased in quails fed containing 200 ppm of licorice root extract and 1% probiotic in the diet.

Table 2 The effects of licorice root on performance parameters

Parameters	Licorice root (%)				SEM <sup>1</sup>	P <sup>2</sup>
	0	0.5	1.0	1.5		
Initial live weight (g)	370.73±4.323	370.68±4.399	370.52±4.347	370.01±4.313	2.156	0.999
Final live weight (g)	368.68±4.037	363.42±5.496	362.04±4.660	368.25±4.831	2.378	0.685
1-4 week period						
Feed Intake (g/bird/day)	41.78±0.122	41.65±0.193	41.65±0.308	41.78±0.100	0.098	0.625
Feed Conversion Ratio (g feed/g egg)	4.04±0.091	4.32±0.136	4.31±0.511	4.09±0.058	0.055	0.159
Egg production (% hen day)	84.74±1.656 <sup>a</sup>	77.82±1.979 <sup>b</sup>	78.34±2.165 <sup>b</sup>	82.36±1.371 <sup>ab</sup>	0.958	0.026
Egg weight (g/bird/day)	10.39±0.231	9.74±0.258	9.72±0.268	10.23±0.142	0.118	0.097
4-8 weeks period						
Feed Intake (g/bird/day)	41.33±0.267	41.92±0.273	41.75±0.511	41.52±0.202	0.165	0.618
Feed Conversion Ratio (g feed/g egg)	4.15±0.090	4.18±0.123	4.30±0.118	4.15±0.068	0.050	0.681
Egg production (% hen day)	83.33±1.847	81.96±2.476	80.53±2.474	83.39±1.637	1.055	0.753
Egg weight (g/bird/day)	10.00±0.206	10.11±0.249	9.79±0.276	10.04±0.166	0.112	0.776
1-8 weeks period						
Feed Intake (g/bird/day)	41.55±0.150	41.78±0.166	41.60±0.294	41.65±0.113	0.096	9.857
Feed Conversion Ratio (g Feed/g egg)	4.10±0.064	4.25±0.091	4.31±0.085	4.12±0.044	0.037	0.134
Egg production (% hen day)	84.04±1.226 <sup>a</sup>	79.89±1.603 <sup>b</sup>	79.43±1.629 <sup>b</sup>	82.87±1.054 <sup>ab</sup>	0.713	0.057
Egg weight (g/bird/day)	10.19±0.156	9.93±0.179	9.75±0.189	10.14±0.109	0.081	0.203

<sup>a,b</sup>: Means within rows with different superscripts differ significantly ( $P<0.05$ ); <sup>1</sup>SEM: Standard error of the mean, <sup>2</sup> Level of significance was set at  $P<0.05$

Egg production ratios were obtained 84.04, 79.89, 79.43 and 82.87% and egg mass value calculated as 8.62, 8.01, 7.84 and 8.43 in control, 0.5, 1.0 and 1.5% licorice root powder supplemented groups, respectively. On the contrary of our study, Awadein et al. (2010) reported that egg production increased with 0.1 and 0.5% licorice addition compared with control group. The same results were obtained by Sedghi et al. (2010a) who reported that diet supplemented with 4 g/kg of licorice extract had greater ( $P<0.06$ ) egg production than the control fed diet during the experiment.

The effects of licorice root powder supplementation on serum parameters of laying quails are shown in Table 3. Licorice root powder supplementation did not significantly affect serum cholesterol, triglyceride and HDL concentration ( $P>0.05$ ). However, compared to control diet, 0.5, 1.0 and 1.5% licorice root powder supplementation decreased triglyceride concentration by 1.38, 11.79 and 21.42%, and increased HDL concentration by 6.37, 18.81 and 23.73%, respectively. Similar results obtained by the Sedghi et al. (2010b) who explained that licorice extracts at the levels of 0.5, 1.0 and 2.0 g/kg decreased cholesterol and triglyceride

concentration and increased HDL concentration in broilers.

Sharifi et al. (2013) reported that the increase in plasma HDL levels may be due to the inhibition of the active enzyme hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA). The enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme in cholesterol biosynthesis that catalyzes the conversion of HMG-CoA (Friesen and Rodwell, 2004). Indeed, it is suggested that medicinal plants cause a decrease in HMG-CoA enzyme synthesis (Yu et al., 1998). Results of the present study for serum cholesterol and triglyceride concentration agree with Al-Daraji, 2012a; Myandoab and Mansoub, 2012 and Rezaei et al., 2014. Myandoab and Mansoub (2012) who reported that the levels of serum cholesterol and triglyceride concentration reduced with licorice root supplementation in quails. In contrast to our results Salary et al. (2014) stated that the inclusion of 0.2 and 0.4% licorice extracts in drinking water of broiler did not decreased triglyceride and cholesterol level.

There was statistically significant difference in serum LDL concentration levels among the experimental groups ( $P<0.05$ ). Licorice root powder supplementation to the

experimental diets decreased the LDL concentration and 1.0 % licorice root powder supplemented group had the lowest LDL level. Results also revealed that the addition of licorice root resulted in significant ( $P<0.05$ ) increase in serum glucose compared with control group. This result is in agreement with findings of Al-Daraji (2012a) and Al-Daraji (2012b). Al-Daraji (2012b) investigated the effect of licorice extract supplemented diet on aflatoxin degradation and blood parameters in broiler chickens. They explained that addition of 150, 300 or 450 mg/kg of licorice root extract to the diet increased plasma glucose level with increasing supplementation ( $P<0.05$ ). In contrast to present study Myandoab and Mansoub (2012) explained that plasma glucose concentration was not significantly different among the groups. Also Sedghi et

al. (2010b) stated that glucose concentration in broiler fed with 0.5, 1.0 and 2.0 g/kg licorice extracts supplementation decreased glucose concentration. Blood glucose level is important in birds. Because glucose is utilized by birds for a variety of functions with the main use being for energy production through cellular oxidation, glycogen synthesis in liver and glycolytic muscles, fatty acid synthesis as well as synthesis of nonessential amino acids, vitamin C, and other metabolites (Braun and Sweaze, 2008). The increase in glucose level in treatment group may be caused by the glycyrrhizin which is active component of licorice root. Glycyrrhizin is a saponin glycoside that is 60 times sweeter than cane sugar (Roshan et al., 2012).

Table 3 The effects of licorice root supplement on plasma biochemical parameters\*

Parameters (mg/dL)	Licorice Root Supplementation, (%)				SEM <sup>1</sup>	P <sup>2</sup>
	0	0.5	1.0	1.5		
Cholesterol	173.45±6.048	167.73±6.717	157.85±4.415	166.73±8.424	3.179	0.365
Triglyceride	1031.50±62.305	1017.28±92.795	909.85±72.148	810.55±49.490	37.354	0.121
LDL	65.36±2.861 <sup>a</sup>	52.20±6.163 <sup>ab</sup>	41.44±7.224 <sup>b</sup>	46.31±6.531 <sup>b</sup>	3.247	0.046
HDL	85.75±5.941	91.22±8.097	101.88±9.324	106.10±8.135	4.058	0.267
Glucose	217.77±9.581 <sup>b</sup>	276.43±10.265 <sup>ab</sup>	293.50±26.908 <sup>a</sup>	300.51±30.857 <sup>a</sup>	11.828	0.047

<sup>a,b</sup>: Means within rows with different superscripts differ significantly ( $P<0.05$ ); <sup>1</sup>SEM: Standard error of the mean, <sup>2</sup> Level of significance was set at  $P<0.05$

Table 4 The effects of licorice root supplement on egg yolk cholesterol level\*

Parameters (mg/g)	Licorice Root Supplementation, (%)				SEM <sup>1</sup>	P <sup>2</sup>
	0	0.5	1.0	1.5		
Egg yolk cholesterol 4 <sup>th</sup> week	17.95±0.554	17.66±0.42	17.40±0.208	16.63±0.287	0.212	0.139
Egg yolk cholesterol 8 <sup>th</sup> week	16.96±0.496	15.87±0.501	15.92±0.430	15.52±0.448	0.249	0.199
Mean egg yolk cholesterol	17.46±0.387	16.76±0.429	16.66±0.335	16.27±0.266	0.188	0.164

<sup>a,b</sup>: Means within rows with different superscripts differ significantly ( $P<0.05$ ); <sup>1</sup>SEM: Standard error of the mean, <sup>2</sup> Level of significance was set at  $P<0.05$

Table 5 Effects of licorice root supplementation on oxidative stress and antioxidant system

Parameters	Licorice Root Supplementation, (%)				SEM <sup>1</sup>	P <sup>2</sup>
	0	0.5	1.0	1.5		
TAS <sup>3</sup> (mmol Trolox equivalents/L)	0.479±0.053 <sup>b</sup>	0.460±0.054 <sup>b</sup>	0.579±0.050 <sup>ab</sup>	0.631±0.019 <sup>a</sup>	0.027	0.072
TOS <sup>4</sup> (µmol H <sub>2</sub> O <sub>2</sub> equivalents /L)	22.42±2.134 <sup>a</sup>	19.489±2.854 <sup>a</sup>	12.191±0.899 <sup>b</sup>	11.97±0.615 <sup>b</sup>	1.350	0.002
OSI <sup>5</sup>	4.924±1.011 <sup>a</sup>	4.771±1.437 <sup>ab</sup>	2.222±0.136 <sup>ab</sup>	1.937±0.103 <sup>b</sup>	0.533	0.058

<sup>a,b</sup>: Means within rows with different superscripts differ significantly ( $P<0.05$ ); <sup>1</sup>SEM: Standard error of the mean, <sup>2</sup> Level of significance was set at  $P<0.05$ ; TAS<sup>3</sup>: Total Antioxidant Status, TOS<sup>4</sup>: Total Oxidant Status, OSI<sup>5</sup>: Oxidative Stress Index

There were no significant differences in egg yolk cholesterol among the groups (Table 4) ( $P>0.05$ ). However, the egg yolk cholesterol levels decreased with increasing level of licorice root powder supplementation. At the 4<sup>th</sup> and 8<sup>th</sup> weeks of the experiment, control group had the highest egg yolk cholesterol content while the lowest egg yolk cholesterol level was determined in the 1.0% supplemented group. Awadein et al. (2010) used 0.1 and 0.5% licorice root as a source of phytoestrogens in the layers diets and stated that egg cholesterol levels were significantly lower in 0.1% and 0.5% licorice supplemented groups than the control group.

Results revealed that quails supplemented with the licorice root powder had a positive effect on the oxidative stress index (OSI), TAS and TOS value. Significant decreases in the TOS values obtained with increasing level of licorice root powder supplementation ( $P<0.05$ ) (Table 5). Thus Haraguchi et al. (2000) stated that

isoflavan derivatives, glabridin (1), hispaglabridin A (2), hispaglabridin B (3), 4'-O-methylglabridin (4) and 3'-hydroxy-4'-O-methylglabridin (5), isolated from *Glycyrrhiza glabra*, were investigated for their ability to protect liver mitochondria against oxidative stresses. Mitochondrial lipid peroxidation linked to respiratory electron transport and that induced non-enzymatically were inhibited by these isoflavans. Vaya et al. (1997) analyzed the antioxidative properties of natural compounds from the root of the plant *Glycyrrhiza glabra* (licorice) toward LDL oxidation. They isolated seven constituents, with antioxidant capacity from *Glycyrrhiza glabra*. The isolated compounds were identified as the isoflavans Hispaglabridin A (1), Hispaglabridin B (4), Glabridin (3), and 4\*-O-Methylglabridin (2), the two chalcones, isoprenylchalcone derivative (5) and Isoliquiritigenin (6), and the isoflavone, Formononetin (7). The antioxidative capacities of the isolated

compounds (1–7) were tested against  $\beta$ -carotene destruction and LDL oxidation. They explained that supplementation of specific licorice constituents to normal human diet, may protect to a certain extent plasma LDL from oxidation.

TAS value results show that the licorice root has antioxidant effect. As a matter of fact, it was determined that the level of total antioxidant increased with increasing levels of licorice root powder supplementation. But there were no significantly differences between treatment groups ( $P>0.05$ ). TAS value was determined as 0.479, 0.460, 0.579 and 0.631 mmol Trolox equivalents/L in the groups containing 0, 0.5, 1.0 and 1.5% licorice root, respectively ( $P>0.05$ ). Similar results were obtained by the (Visavadiya and Narasimhacharya, 2006; Sen et al., 2011; Zhao et al., 2011; Habibi et al., 2014) who found that licorice root has antioxidant capacity. Visavadiya and Narasimhacharya (2006) investigated the hypocholesterolaemic and antioxidant effects of *Glycyrrhiza glabra* root powder in hypercholesterolaemic male albino rats. They reported that *Glycyrrhiza glabra* root powder administration to hypercholesterolaemic rats decreased hepatic lipid peroxidation with a concomitant increase in superoxide dismutase (SOD) and catalase activities and total ascorbic acid content. *Glycyrrhiza glabra* root powder increased hepatic HMG-CoA reductase activity in rats supplemented with 5 and 10 gm% level. Sen et al. (2011) stated that oxidative stress parameters, namely, serum superoxide dismutase, catalase, malondialdehyde in diabetic rats were reverted to respective normal values after glycyrrhizin administration.

The results of the present study suggested that the licorice root powder could be used in laying quail diets without negative effect on performance. Licorice root powder could be used in quail diets to increase antioxidant capacity and used as a feed additive to lower cholesterol levels in quail eggs.

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